

EXHIBIT A

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CLEAN COPY OF THE CLAIMS FOLLOWING ENTRY
AMENDMENT FILED MARCH 4, 2003
U.S. PATENT APPLICATION NO. 09/823,307
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71. (Three times amended) A monoclonal antibody that recognizes a human 8F4 polypeptide, wherein said 8F4 polypeptide:

- a) is an inducible T cell costimulatory molecule;
- b) occurs on two-signal-activated human T lymphocytes;
- c) exhibits a molecular weight of about 55 to 60 kilodaltons as determined by non-reducing sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE); and
- d) is a dimer of two peptide chains exhibiting molecular weights of about 27 kilodaltons and 29 kilodaltons, as measured by reducing SDS-PAGE,

wherein the human 8F4 polypeptide is recognized by the antibody deposited with the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH ("DSMZ") and assigned accession no. DSM ACC2539;

and wherein the monoclonal antibody, in conjunction with anti-CD3 monoclonal antibody OKT3, costimulates proliferation of human T lymphocytes.

72. The monoclonal antibody of claim 71, wherein said monoclonal antibody recognizes the human 8F4 polypeptide of about 55 kilodaltons to 60 kilodaltons, as determined by non-reducing SDS-PAGE.

73. The monoclonal antibody of claim 71, wherein said monoclonal antibody recognizes the peptide chain of about 27 kilodaltons, as determined by reducing SDS-PAGE.

74. The monoclonal antibody of claim 71, wherein said monoclonal antibody recognizes the peptide chain of about 29 kilodaltons, as determined by reducing SDS-PAGE.

75. The monoclonal antibody of claim 71, wherein said monoclonal antibody recognizes a human 8F4 polypeptide present on activated human CD4⁺ T lymphocytes and activated human CD8⁺ T lymphocytes.

76. (Four times amended) A monoclonal antibody that recognizes a human 8F4 polypeptide, wherein said 8F4 polypeptide:

- a) is an inducible T cell costimulatory molecule;
- b) occurs on two-signal-activated human T lymphocytes;
- c) exhibits a molecular weight of about 55 to 60 kilodaltons as determined by non-reducing sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE); and
- d) is a dimer of two peptide chains exhibiting molecular weights of about 27 kilodaltons and 29 kilodaltons, as measured by reducing SDS-PAGE,

wherein the human 8F4 polypeptide is recognized by the antibody deposited with the DSMZ and assigned accession no. DSM ACC2539;

wherein the monoclonal antibody, in conjunction with anti-CD3 monoclonal antibody OKT3, costimulates proliferation of human T lymphocytes;

and wherein the monoclonal antibody inhibits costimulation of T lymphocytes by the human 8F4 polypeptide.

78. (Three times amended) A hybridoma that produces a monoclonal antibody that recognizes a human 8F4 polypeptide, wherein said 8F4 polypeptide:

- a) is an inducible T cell costimulatory molecule;
- b) occurs on two-signal-activated human T lymphocytes;
- c) exhibits a molecular weight of about 55 to 60 kilodaltons as determined by non-reducing sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE); and
- d) is a dimer of two peptide chains exhibiting molecular weights of about 27 kilodaltons and 29 kilodaltons, as measured by reducing SDS-PAGE,

wherein the human 8F4 polypeptide is recognized by the antibody deposited with the DSMZ and assigned accession no. DSM ACC2539;

and wherein the hybridoma produces a monoclonal antibody that, in conjunction with anti-CD3 monoclonal antibody OKT3, costimulates proliferation of human T lymphocytes.

79. The hybridoma of claim 78, wherein said hybridoma produces a monoclonal antibody that recognizes the human 8F4 polypeptide of about 55 kilodaltons to 60 kilodaltons, as determined by non-reducing SDS-PAGE.

80. The hybridoma of claim 78, wherein said hybridoma produces a monoclonal antibody that recognizes the peptide chain of about 27 kilodaltons, as determined by reducing SDS-PAGE.

81. The hybridoma of claim 78, wherein said hybridoma produces a monoclonal antibody that recognizes the peptide chain of about 29 kilodaltons, as determined by reducing SDS-PAGE.

82. The hybridoma of claim 78, wherein said hybridoma produces a monoclonal antibody that recognizes a human 8F4 polypeptide present on activated human CD4+ T lymphocytes and activated human CD8+ T lymphocytes.

83. (Four times amended) A hybridoma that produces a monoclonal antibody that recognizes a human 8F4 polypeptide, wherein said 8F4 polypeptide:

- a) is an inducible T cell costimulatory molecule;
- b) occurs on two-signal-activated human T lymphocytes;
- c) exhibits a molecular weight of about 55 to 60 kilodaltons as determined by non-reducing sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE); and
- d) is a dimer of two peptide chains exhibiting molecular weights of about 27 kilodaltons and 29 kilodaltons, as measured by reducing SDS-PAGE,

wherein the human 8F4 polypeptide is recognized by the antibody deposited with the DSMZ and assigned accession no. DSM ACC2539;

wherein the hybridoma produces a monoclonal antibody,

wherein the monoclonal antibody, in conjunction with anti-CD3 monoclonal antibody OKT3, costimulates proliferation of human T lymphocytes;

and wherein the monoclonal antibody inhibits costimulation of T lymphocytes by the human 8F4 polypeptide.

85. (Three times amended) A pharmaceutical composition comprising a monoclonal antibody that recognizes a human 8F4 polypeptide, wherein said 8F4 polypeptide:

- a) is an inducible T cell costimulatory molecule;
- b) occurs on two-signal-activated human T lymphocytes;
- c) exhibits a molecular weight of about 55 to 60 kilodaltons as determined by non-reducing sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE); and
- d) is a dimer of two peptide chains exhibiting molecular weights of about 27 kilodaltons and 29 kilodaltons, as measured by reducing SDS-PAGE,

wherein the human 8F4 polypeptide is recognized by the antibody deposited with the DSMZ and assigned accession no. DSM ACC2539;

and wherein the pharmaceutical composition comprises a monoclonal antibody that, in conjunction with anti-CD3 monoclonal antibody OKT3, costimulates proliferation of human T lymphocytes.

86. (Four times amended) A pharmaceutical composition comprising a monoclonal antibody that recognizes a human 8F4 polypeptide, wherein said 8F4 polypeptide:

- a) is an inducible T cell costimulatory molecule;
- b) occurs on two-signal-activated human T lymphocytes;
- c) exhibits a molecular weight of about 55 to 60 kilodaltons as determined by non-reducing sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE); and
- d) is a dimer of two peptide chains exhibiting molecular weights of about 27 kilodaltons and 29 kilodaltons, as measured by reducing SDS-PAGE,

wherein the human 8F4 polypeptide is recognized by the antibody deposited with the DSMZ and assigned accession no. DSM ACC2539;
and wherein the pharmaceutical composition comprises a monoclonal antibody;
wherein the monoclonal antibody, in conjunction with anti-CD3 monoclonal antibody OKT3, costimulates proliferation of human T lymphocytes;
and wherein the monoclonal antibody inhibits costimulation of T lymphocytes by the human 8F4 polypeptide.

88. (Amended) A method for producing the monoclonal antibody of claim 71 or 76, comprising: culturing an antibody-secreting hybridoma obtained by:
(i) fusion of a myeloma cell line cell with a spleen cell of a mouse immunized with 2-signal-activated human T lymphocytes; and
(ii) selection of a hybridoma that produces said antibody,
such that the monoclonal antibody is produced.

89. (Amended) The monoclonal antibody of claim 71 or 76, wherein said monoclonal antibody recognizes the human 8F4 polypeptide of about 55 kilodaltons to 60 kilodaltons, as determined by non-reducing SDS-PAGE.

90. (Amended) The monoclonal antibody of claim 71 or 76, wherein said monoclonal antibody recognizes the peptide chain of about 27 kilodaltons, as determined by reducing SDS-PAGE.

91. (Amended) The monoclonal antibody of claim 71 or 76, wherein said monoclonal antibody recognizes the peptide chain of about 29 kilodaltons, as determined by reducing SDS-PAGE.

92. (Amended) The monoclonal antibody of claim 71 or 76, wherein said monoclonal antibody recognizes a human 8F4 polypeptide present on activated human CD4⁺ T lymphocytes and activated human CD8⁺ T lymphocytes.

93. (Amended) The hybridoma of claim 78 or 83, wherein said hybridoma produces a monoclonal antibody that recognizes the human 8F4 polypeptide of about 55 kilodaltons to 60 kilodaltons, as determined by non-reducing SDS-PAGE.

94. (Amended) The hybridoma of claim 78 or 83, wherein said hybridoma produces a monoclonal antibody that recognizes the peptide chain of about 27 kilodaltons, as determined by reducing SDS-PAGE.

95. (Amended) The hybridoma of claim 78 or 83, wherein said hybridoma produces a monoclonal antibody that recognizes the peptide chain of about 29 kilodaltons, as determined by reducing SDS-PAGE.

96. (Amended) The hybridoma of claim 78 or 83, wherein said hybridoma produces a monoclonal antibody that recognizes a human 8F4 polypeptide present on activated human CD4⁺ T lymphocytes and activated human CD8⁺ T lymphocytes.

97. (Three times amended) A method of producing a human 8F4 polypeptide-specific monoclonal antibody, comprising: culturing an antibody-secreting hybridoma obtained by (i) fusion of a myeloma cell line cell with a spleen cell of a mouse immunized with an antigen comprising a human 8F4 polypeptide and (ii) selection of a hybridoma that produces said antibody, wherein said human 8F4 polypeptide:

- a) is an inducible T cell costimulatory molecule;
- b) occurs on two-signal-activated human T lymphocytes;
- c) exhibits a molecular weight of about 55 to 60 kilodaltons as determined by non-reducing sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE); and
- d) is a dimer of two peptide chains exhibiting molecular weights of about 27 kilodaltons and 29 kilodaltons, as measured by reducing SDS-PAGE,

and wherein the human 8F4 polypeptide is recognized by the antibody deposited with the DSMZ and assigned accession no. DSM ACC2539;
such that the monoclonal antibody is produced.

98. A monoclonal antibody produced by the method of claim 97.
99. (Amended) A hybridoma cell line deposited with the DSMZ and assigned accession no. DSM ACC2539.
100. A monoclonal antibody 8F4 produced by the hybridoma cell line of claim 99.
101. The monoclonal antibody of claim 76, wherein the monoclonal antibody inhibition of costimulation of T lymphocytes by the human 8F4 polypeptide is exhibited as an inhibition of 8F4-induced T lymphocyte proliferation.
102. The monoclonal antibody of claim 76, wherein the monoclonal antibody inhibition of costimulation of T lymphocytes by the human 8F4 polypeptide is exhibited as an inhibition of 8F4-induced ATAC expression.
103. The monoclonal antibody of claim 76, wherein the monoclonal antibody inhibition of costimulation of T lymphocytes by the human 8F4 polypeptide is exhibited as an inhibition of 8F4-induced CD25 expression.
104. The monoclonal antibody of claim 76, wherein the monoclonal antibody inhibition of costimulation of T lymphocytes by the human 8F4 polypeptide is exhibited as an inhibition of 8F4-induced CD69 expression.
105. The monoclonal antibody of claim 76, wherein the monoclonal antibody inhibition of costimulation of T lymphocytes by the human 8F4 polypeptide is exhibited as an inhibition of 8F4-induced TRAP expression.
106. The monoclonal antibody of claim 76, wherein the monoclonal antibody inhibition of costimulation of T lymphocytes by the human 8F4 polypeptide is exhibited as an inhibition of T lymphocyte induction of immunoglobulin production by B lymphocytes.

107. The monoclonal antibody of claim 76, wherein the monoclonal antibody inhibition of costimulation of T lymphocytes by the human 8F4 polypeptide is exhibited as an inhibition of 8F4-induced reduction of apoptosis in activated T lymphocytes.

108. The hybridoma of claim 83, wherein the monoclonal antibody inhibition of costimulation of T lymphocytes by the human 8F4 polypeptide is exhibited as an inhibition of 8F4-induced T lymphocyte proliferation.

109. The hybridoma of claim 83, wherein the monoclonal antibody inhibition of costimulation of T lymphocytes by the human 8F4 polypeptide is exhibited as an inhibition of 8F4-induced ATAC expression.

110. The hybridoma of claim 83, wherein the monoclonal antibody inhibition of costimulation of T lymphocytes by the human 8F4 polypeptide is exhibited as an inhibition of 8F4-induced CD25 expression.

111. The hybridoma of claim 83, wherein the monoclonal antibody inhibition of costimulation of T lymphocytes by the human 8F4 polypeptide is exhibited as an inhibition of 8F4-induced CD69 expression.

112. The hybridoma of claim 83, wherein the monoclonal antibody inhibition of costimulation of T lymphocytes by the human 8F4 polypeptide is exhibited as an inhibition of 8F4-induced TRAP expression.

113. The hybridoma of claim 83, wherein the monoclonal antibody inhibition of costimulation of T lymphocytes by the human 8F4 polypeptide is exhibited as an inhibition of T lymphocyte induction of immunoglobulin production by B lymphocytes.

114. The hybridoma of claim 83, wherein the monoclonal antibody inhibition of costimulation of T lymphocytes by the human 8F4 polypeptide is exhibited as an inhibition of 8F4-induced reduction of apoptosis in activated T lymphocytes.

115. The pharmaceutical composition of claim 86, wherein the monoclonal antibody inhibition of costimulation of T lymphocytes by the human 8F4 polypeptide is exhibited as an inhibition of 8F4-induced T lymphocyte proliferation.

116. The pharmaceutical composition of claim 86, wherein the monoclonal antibody inhibition of costimulation of T lymphocytes by the human 8F4 polypeptide is exhibited as an inhibition of 8F4-induced ATAC expression.

117. The pharmaceutical composition of claim 86, wherein the monoclonal antibody inhibition of costimulation of T lymphocytes by the human 8F4 polypeptide is exhibited as an inhibition of 8F4-induced CD25 expression.

118. The pharmaceutical composition of claim 86, wherein the monoclonal antibody inhibition of costimulation of T lymphocytes by the human 8F4 polypeptide is exhibited as an inhibition of 8F4-induced CD69 expression.

119. The pharmaceutical composition of claim 86, wherein the monoclonal antibody inhibition of costimulation of T lymphocytes by the human 8F4 polypeptide is exhibited as an inhibition of 8F4-induced TRAP expression.

120. The pharmaceutical composition of claim 86, wherein the monoclonal antibody inhibition of costimulation of T lymphocytes by the human 8F4 polypeptide is exhibited as an inhibition of T lymphocyte induction of immunoglobulin production by B lymphocytes.

121. The pharmaceutical composition of claim 86, wherein the monoclonal antibody inhibition of costimulation of T lymphocytes by the human 8F4 polypeptide is exhibited as an inhibition of 8F4-induced reduction of apoptosis in activated T lymphocytes.

122. The monoclonal antibody of claim 76, wherein the monoclonal antibody inhibition of costimulation of T lymphocytes by the human 8F4 polypeptide is exhibited as an inhibition of: 8F4-induced T lymphocyte proliferation, 8F4-induced ATAC expression, 8F4-induced CD25 expression, 8F4-induced CD69 expression, 8F4-induced TRAP expression, T lymphocyte induction of immunoglobulin production by B lymphocytes and 8F4-induced reduction of apoptosis in activated T lymphocytes.

123. The hybridoma of claim 83, wherein the monoclonal antibody inhibition of costimulation of T lymphocytes by the human 8F4 polypeptide is exhibited as an inhibition of: 8F4-induced T lymphocyte proliferation, 8F4-induced ATAC expression, 8F4-induced CD25 expression, 8F4-induced CD69 expression, 8F4-induced TRAP expression, T lymphocyte induction of immunoglobulin production by B lymphocytes and 8F4-induced reduction of apoptosis in activated T lymphocytes.

124. The pharmaceutical composition of claim 86, wherein the monoclonal antibody inhibition of costimulation of T lymphocytes by the human 8F4 polypeptide is exhibited as an inhibition of: 8F4-induced T lymphocyte proliferation, 8F4-induced ATAC expression, 8F4-induced CD25 expression, 8F4-induced CD69 expression, 8F4-induced TRAP expression, T lymphocyte induction of immunoglobulin production by B lymphocytes and 8F4-induced reduction of apoptosis in activated T lymphocytes.

125. The monoclonal antibody of claim 71, wherein the costimulation of proliferation of human T lymphocytes is exhibited at a dilution of OKT-3 of 1:10,000.

126. The monoclonal antibody of claim 71, wherein the costimulation of proliferation of human T lymphocytes is exhibited at a dilution of OKT-3 of 1:5,000.

127. The monoclonal antibody of claim 71, wherein the costimulation of proliferation of human T lymphocytes is exhibited at a dilution of OKT-3 of 1:2,500.

128. The monoclonal antibody of claim 71, wherein the costimulation of proliferation of human T lymphocytes is exhibited at a dilution of OKT-3 of 1:1,000.

129. The monoclonal antibody of claim 76, wherein the costimulation of proliferation of human T lymphocytes is exhibited at a dilution of OKT-3 of 1:10,000.

130. The monoclonal antibody of claim 76, wherein the costimulation of proliferation of human T lymphocytes is exhibited at a dilution of OKT-3 of 1:5,000.

131. The monoclonal antibody of claim 76, wherein the costimulation of proliferation of human T lymphocytes is exhibited at a dilution of OKT-3 of 1:2,500.

132. The monoclonal antibody of claim 76, wherein the costimulation of proliferation of human T lymphocytes is exhibited at a dilution of OKT-3 of 1:1,000.

133. The hybridoma of claim 78, wherein the costimulation of proliferation of human T lymphocytes is exhibited at a dilution of OKT-3 of 1:10,000.

134. The hybridoma of claim 78, wherein the costimulation of proliferation of human T lymphocytes is exhibited at a dilution of OKT-3 of 1:5,000.

135. The hybridoma of claim 78, wherein the costimulation of proliferation of human T lymphocytes is exhibited at a dilution of OKT-3 of 1:2,500.

136. The hybridoma of claim 78, wherein the costimulation of proliferation of human T lymphocytes is exhibited at a dilution of OKT-3 of 1:1,000.

137. The hybridoma of claim 83, wherein the costimulation of proliferation of human T lymphocytes is exhibited at a dilution of OKT-3 of 1:10,000.

138. The hybridoma of claim 83, wherein the costimulation of proliferation of human T lymphocytes is exhibited at a dilution of OKT-3 of 1:5,000.

139. The hybridoma of claim 83, wherein the costimulation of proliferation of human T lymphocytes is exhibited at a dilution of OKT-3 of 1:2,500.

140. The hybridoma of claim 83, wherein the costimulation of proliferation of human T lymphocytes is exhibited at a dilution of OKT-3 of 1:1,000.

141. The pharmaceutical composition of claim 85, wherein the costimulation of proliferation of human T lymphocytes is exhibited at a dilution of OKT-3 of 1:10,000.

142. The pharmaceutical composition of claim 85, wherein the costimulation of proliferation of human T lymphocytes is exhibited at a dilution of OKT-3 of 1:5,000.

143. The pharmaceutical composition of claim 85, wherein the costimulation of proliferation of human T lymphocytes is exhibited at a dilution of OKT-3 of 1:2,500.

144. The pharmaceutical composition of claim 85, wherein the costimulation of proliferation of human T lymphocytes is exhibited at a dilution of OKT-3 of 1:1,000.

145. The pharmaceutical composition of claim 86, wherein the costimulation of proliferation of human T lymphocytes is exhibited at a dilution of OKT-3 of 1:10,000.

146. The pharmaceutical composition of claim 86, wherein the costimulation of proliferation of human T lymphocytes is exhibited at a dilution of OKT-3 of 1:5,000.

147. The pharmaceutical composition of claim 86, wherein the costimulation of proliferation of human T lymphocytes is exhibited at a dilution of OKT-3 of 1:2,500.

148. The pharmaceutical composition of claim 86, wherein the costimulation of proliferation of human T lymphocytes is exhibited at a dilution of OKT-3 of 1:1,000.

149. (New) A human 8F4 polypeptide, wherein said 8F4 polypeptide:

- a) is an inducible T cell costimulatory molecule;
- b) occurs on two-signal-activated human T lymphocytes;
- c) exhibits a molecular weight of about 55 to 60 kilodaltons as determined by non-reducing sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE);
- d) is a dimer of two peptide chains exhibiting molecular weights of about 27 kilodaltons and 29 kilodaltons, as measured by reducing SDS-PAGE; and
- e) is recognized by the antibody deposited with the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH ("DSMZ") and assigned accession no. DSM ACC2539.

150. (New) An isolated human 8F4 polypeptide, wherein said 8F4 polypeptide:

- a) is an inducible T cell costimulatory molecule;
- b) occurs on two-signal-activated human T lymphocytes;
- c) exhibits a molecular weight of about 55 to 60 kilodaltons as determined by non-reducing sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE);
- d) is a dimer of two peptide chains exhibiting molecular weights of about 27 kilodaltons and 29 kilodaltons, as measured by reducing SDS-PAGE; and
- e) is recognized by the antibody deposited with the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH ("DSMZ") and assigned accession no. DSM ACC2539.

151. (New) An isolated human 8F4 polypeptide, wherein said 8F4 polypeptide:

- a) is an inducible T cell costimulatory molecule;
- b) occurs on two-signal-activated human T lymphocytes;

- c) exhibits a molecular weight of about 55 to 60 kilodaltons as determined by non-reducing sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE); and
- d) is recognized by the antibody deposited with the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH ("DSMZ") and assigned accession no. DSM ACC2539.

152. (New) An isolated peptide chain exhibiting a molecular weight of about 27 kilodaltons as measured by reducing SDS-PAGE, which peptide chain is a chain of the polypeptide of claim 151.

153. (New) An isolated peptide chain exhibiting a molecular weight of about 29 kilodaltons as measured by reducing SDS-PAGE, which peptide chain is a chain of the polypeptide of claim 151.